**Amendments to the Specification:**

## AMENDMENT TO THE SPECIFICATION

## IN THE TITLE

*Please amend the title as follows:*

Hierarchical Optimization for Procedural Effectiveness in Diagnosing Mutations That Relate to Disease

## IN THE SPECIFICATION

***On page 1, lines 16-19, please amend as follows (Please note that the underlining of the reference should be deleted, not the reference):***

For example, in both Duchenne Muscular Dystrophy and Becker Muscular Dystrophy, mutations cluster in two recombination "hot spots" ~~[Den Dunnen et al 1989]~~. (Den Dunnen et al 1989). Similarly, the most common genetic defect that causes cystic fibrosis (ΔF508) accounts for about 30-80% of mutant alleles depending on the ethnic group [CF Genotype-Phenotype Consortium1993].

***On page 3, lines 2-11, please amend as follows:***

This invention provides a computer readable medium having computer-executable instructions that when executed by a computer cause the computer to perform a method for determining an optimal test order for diagnosing mutations that relate to a disease, the method comprising the steps of receiving data indicative of a historical frequency distribution of mutations that relate to the disease and the assays required to ~~diagnosies~~ diagnose the disease, creating a history database, the database comprising a sequence of records based on the data, receiving new data indicative of the historical frequency distribution of mutations that relate to the disease and the assays required to ~~diagnosies~~ diagnose the disease, applying at least one

decision tree algorithm, wherein the at least one decision tree algorithm scores at least a portion of the new data and generating a recommendation if the score satisfies a threshold.

***On page 4, lines 3-14, please amend as follows:***

In one embodiment the at least one decision tree algorithm further determines a projected cost for each test associated with the optimal test order if the score satisfies a threshold, wherein the projected cost is presented via the output device. In another embodiment the received information comprises the historical frequency distribution of mutations that relate to the disease and the assays required to ~~diagnosis~~ diagnose the disease. In yet another embodiment the at least one decision tree algorithm is specific to hereditary diseases selected from the group consisting of breast cancer, colorectal cancer, lung cancer, prostate cancer, retinoblastoma, and hereditary hemorrhagic telangiectasia. In another embodiment the decision tree consists of at least two strategies. In yet another embodiment the at least two strategies are ranked by projected cost and in yet another embodiment the at least two strategies comprise at least two assays. In one embodiment, the at least two strategies are ranked based on minimum projected cost to perform the at least two medical diagnostic assays.

***On page 4, lines 15- page 5, lines 3, please amend as follows:***

The invention also relates to a method of determining the optimal test order for diagnosing mutations that relate to a disease, comprising the steps of receiving data indicative of a historical frequency distribution of mutations that relate to the disease and the assays required to ~~diagnosis~~ diagnose the disease; creating a history database, the database comprising a sequence of records based on the data; receiving new data indicative of the historical frequency distribution of mutations that relate to the disease and the assays required to diagnosis the disease; applying at least one decision tree algorithm, wherein the at least one decision tree algorithm scores at least a portion of the new data; and generating a recommendation if the score satisfies a threshold. In one embodiment, applying at least one decision tree algorithm comprises the steps of accessing a set of records within the history database; generating at least two strategies from the accessed records; comparing the at least two strategies against

each other; and calculating the projected cost for the at least one strategy identified by the comparing step. In yet another embodiment, the projected cost is calculated from a preselected minimum number of records within the history database.

***On page 9, line 1, please add the following:***

This figure depicts a decision tree for comparing two assays (i and j), given one of three prior search histories, shown here as entry points, No Prior Information, Partial Heterozygous, and Partial Homozygous. Table 1 counts paths and costs along each path, and defines the notations used in the figure.

***On page 36, line 3-20, please amend as follows:***

QM-PCR was used to screen for changes in exon size and copy number. All 27 exons of the gene were amplified using intronic primers designed to include splice sites. The 3' end of the promoter, containing binding sites for ATF, E2F, SP1, AP1 and HRE elements [Gill, 1994], was amplified as a single fragment. Amplification was performed in six multiplex sets containing between 1 and 8 fluorescent-labelled primer pairs (Cy 5.5) that yielded products of different sizes to allow simultaneous visualization of fragments. Reactions were performed with AmpliTaq DNA Polymerase kit (Applied Biosystems, Foster City, CA). PCR conditions were optimized so that each exon was amplified quantitatively. Either a 282 bp or a 329 bp product from exon 4 of the human retinaldehyde binding protein gene (chromosome 15) was used as internal control for quantitation against external controls known to have the following RB1 status: nullisomic, WERI-RB1 retinoblastoma cell line [McFall, 1977]; monosomic, EL cell line [Benedict, 1983]; and diploid, normal. Amplified products were heat denatured, separated on a 6% polyacrylamide gel and analyzed using the OpenGene® Automated DNA System (Visible Genetics Inc, Toronto). Fragment detection and subsequent calculations were performed by the Gene Objects 3.1 software (Visible Genetics Inc, Toronto). Ratios of RB1 derived peaks to internal control peaks were used to calculate gene copy number. Size standards labelled with Cy5.5 were used to identify aberrantly migrating bands indicating suspect intra-exonic insertion or deletion, which were verified and further characterized by bi-directional sequencing.

***On page 37, line 7-18, please amend as follows:***

Duplex PCR reactions that amplify pairs of exons were performed with AmpliTaq DNA Polymerase kit (Applied Biosystems, Foster City, CA). Each pair of exons was sequenced simultaneously using the Cy5/Cy5.5 Dye Primer Cycle Sequencing Kit (Amersham, Quebec/Visible Genetics Inc, Toronto). Each primer mixture contained two primers, each labelled with either Cy5 or Cy5.5, targeting exons amplified in the preceding duplex PCR. Primers were designed to include on average 50 base pairs of intronic sequence and therefore included traditionally recognized splice sites. Exons were duplexed based on similar mutation yields (to load early tests with positive results) or compatible reaction conditions. To sequence all 27 RB1 exons and promoter region, a total of 14 duplex sequencing reactions were performed. Sequences were analyzed and compared to wild type RB1 (Genebank Accession L11910) using the OpenGene® Automated DNA System and Gene Librarian Software, Version 3.1 (Visible Genetics Inc., Toronto) to detect sequence alterations.